Fourier Transform Infrared Quantitative Analysis of Sugars and Lignin in Pretreated Softwood Solid Residues

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Abstract

Hydrolysates were obtained from dilute sulfuric acid pretreatment of whole-tree softwood forest thinnings and softwood sawdust. Mid-infrared (IR) spectra were obtained on sample sets of wet washed hydrolysates, and 45°C vacuum-dried washed hydrolysates, using a Fourier transform infrared (FTIR) spectrophotometer equipped with a diamond-composite attenuated total reflectance (ATR) cell. Partial least squares (PLS) analysis of spectra from each sample set was performed. Regression analyses for sugar components and lignin were generated using results obtained from standard wet chemical and high-performance liquid chromatography methods. The correlation coefficients of the predicted and measured values were >0.9. The root mean square standard error of the estimate for each component in the residues was generally within 2 wt% of the measured value except where reported in the tables. The PLS regression analysis of the wet washed solids was similar to the PLS regression analysis on the 45°C vacuum-dried sample set. The FTIR-ATR technique allows mid-IR spectra to be obtained in a few minutes from wet washed or dried washed pretreated biomass solids. The prediction of the solids composition of an unknown washed pretreated solid is very rapid once the PLS method has been calibrated with known standard solid residues.

Index Entries: Fourier transform infrared; biomass; softwood; dilute-acid pretreatment; acid hydrolysis.

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Introduction

The conversion of renewable lignocellulosic resources to bioethanol requires a number of process steps. We have used dilute-acid pretreatment with sulfuric acid as one of the early process steps to hydrolyze a significant portion of the hemicellulose in softwood feedstocks consisting of whole-tree forest thinnings or sawdust from sawmill wastes. A second pretreatment at higher temperatures and acid concentrations was used to hydrolyze the remaining hemicellulose and some of the cellulose from the first-stage solid residues. The extent of the pretreatment reactions must be controlled and optimized for each feedstock to maximize the production of bioethanol. In conversion processes requiring enzymatic hydrolysis of the residual cellulose, the pretreatment steps may increase the susceptibility of the remaining cellulose to hydrolysis by cellulase enzymes resulting in increased yields of sugars or ethanol.

First- and second-stage pretreatment of softwood feedstocks with dilute sulfuric acid is usually a short process step involving only a few minutes at high temperature in the reactor. Controlling the reactions requires rapid and accurate methods of analysis for monitoring the reactor. Conventional methods such as wet chemical analysis and high-performance liquid chromatography are time-consuming and too slow to be useful for the control of the reactor. The standard wet chemical method for solids compositional analysis *(1)* may take several days to complete. Although this method is quite accurate, it is too slow to enable control of the short dilute-acid pretreatment step. However, if a rapid method to monitor and control the pretreatment reactor is available, ethanol production can be increased. Fourier transform infrared (FTIR) spectroscopy is rapid and quantitative, and coupled with advanced high-temperature, high-pressure attenuated total reflectance (ATR) probes the capability of monitoring pretreatment reactors is possible.

FTIR spectroscopic analysis is a rapid and nondestructive technique for the qualitative and quantitative identification of components in solids in the mid-IR region *(2)*. The usefulness of FTIR spectroscopy of solids has increased as sampling device technology has advanced. The FTIR analysis of wet solid residues, such as pretreated whole slurries or washed solids from those slurries, has been severely restricted in the past because of the high IR background absorbance of water. Extremely short pathlength cells (not practical for analyzing high-solids slurries) were needed to obtain transmission spectra *(3)*. However, in the ATR mode *(4–7)*, the high background absorbance caused by water in pretreated slurries and washed solids can be partially controlled by choosing ATR cells incorporating single or multiple reflections within the crystal. This allows attenuation of the incident radiation by the insoluble fiber to give the mid-IR spectra without the high water background absorbance completely obscuring the spectra. Recent developments in ATR cells and probes have extended the application of mid-IR spectroscopy to the qualitative and quantitative analysis of wet and abrasive solid residue *(8)*.

Diffuse reflectance IR Fourier transform spectroscopic methods or transmission spectroscopy utilizing KBr pellets previously has been used to study whole wood, pretreated wood, wood surfaces, lignin, and pulp and paper substrates *(9–17)*. However, the use of FTIR-ATR spectroscopy to study solids was severely limited because of the soft ATR crystals (i.e., ZnSe, ZnS, and KRS-5) then available. Recent availability of ATR cells utilizing very hard crystals of silicon and diamond has overcome this difficulty. These crystals possess considerable hardness and chemical inertness; thus, the samples can be pressed onto the ATR crystal at high pressures, allowing a more uniform penetration by the evanescent radiation *(5)* and a higher degree of reproducibility. The recent development of diamond-composite ATR cells has extended FTIR spectroscopy into areas of research not possible before because the diamond is highly chemically inert, exhibits high hardness and mechanical strength, and is optically transparent in the visible and most of the mid-IR region. Because a diamond surface has the lowest coefficient of friction of all the available crystal materials, few materials stick to this surface *(8)*. Diamond-composite ATR probes are now available that work at temperatures as high as 250°C and at 1500 psi (10 MPa).

In the present study we used a diamond-composite ATR cell and an FTIR spectrometer to obtain mid-IR spectra of washed pretreated softwood solid residues, and washed and dried pretreated softwood solid residues. The partial least squares (PLS) option in the commercially available software package TQ Analyst™ (Nicolet Instruments, Madison, WI) was used to regress the spectra into methods capable of predicting the glucan, mannan, galactan, xylan, and lignin compositions of pretreated softwood solid residues. Using a calibrated method, FTIR-ATR can rapidly determine (in a few minutes) the solids composition of washed residues from a pretreatment reactor.

Materials and Methods

Preparation and Pretreatment of Feedstock

Whole-tree forest thinning was obtained by the Pacific Wood Fuels Company, Redding, CA, from a site near Quincy, CA, and prepared as previously reported *(18)*. Pretreatment of this whole-tree forest thinnings feedstock for first- and second-stage experiments was performed using a 4-L steam explosion reactor (NREL Digester) as described earlier *(19)*. Conditions ranged from 180 to 215°C, 0.35 to 2.5% (w/w) sulfuric acid, and 120-s to 9-min residence times.

Barrels of typical sawdusts from southeastern Alaska were obtained from sawmills of Ketchikan Pulp, by Sealaska of Juneau, Alaska. The barrels of fresh sawdusts were rapidly shipped to NREL to minimize degradation in transit. A mixture of 62% (dry wt basis) hemlock, 26% Sitka spruce, and 12% red cedar sawdusts was prepared by mixing four times on a large tarpaulin using the method of coning and quartering. The composition of

mixed sawdusts was chosen to represent the statistical species populations of softwood trees in the forests of southeastern Alaska. Each barrel of sawdust was obtained from a different sawmill conducting a campaign utilizing an individual softwood species for a particular forest product or customer. Samples of this sawdust mixture were pretreated in the 4-L steam explosion reactor. First-stage pretreatment conditions varied from 180 to 190°C, 3 to 4 min, and 0.7% (w/w) H_2SO_4. Second-stage pretreatment conditions varied from 205 to 215°C, 3- to 4-min residence times, and 0.7 to 1% (w/w) H_2SO_4.

Pretreated slurry samples were extensively washed by centrifugation before obtaining mid-IR spectra of the wet solids. Representative samples (10 g) of the pretreated slurries were suspended in 40 mL of deionized-glass distilled water and centrifuged at 6000g for 5 min, and the supernatants were discarded. The washing procedure was repeated a minimum of five times. The percentage of moisture of the washed samples was obtained by standard oven-drying methods at 105°C. Washed and dried solids were prepared from the wet washed solids by drying under vacuum at 45°C for 3 d. Representative samples of the wet washed and dried washed solid samples were ground using an agate mortar and pestle before application to the diamond surface of the ATR cell. Grinding was minimal (usually <30 s on a 0.5-g sample) to prevent the solids from being degraded by the heat of grinding.

IR Spectroscopy

Approximately 50–100 mg of the ground sample was required to fill the sample well of the diamond-composite ATR sample cell. The sample cell was equipped with a spring-loaded anvil to reproducibly press the solid sample uniformly and tightly against the diamond surface. Mid-IR spectra were obtained by averaging 512 scans from 4000 to 400 cm^{-1}, at 2-cm^{-1} resolution using a Nicolet Avatar 360® FTIR spectrometer (Nicolet Instrument) and a six-reflection diamond-composite ASI DurasamplIR™ ATR cell (ASI SensIR Technologies, Danbury, CT).

PLS Method

PLS calibration methods for solid compositions were determined using the PLS regression analysis option in TQ Analyst™ (version 6.0) on 35 wet or 34 dried solid sample spectra. Wet chemical results for percentage of solids, percentage of sugars, and Klason and acid-soluble lignin for each sample were entered into the software package spreadsheet before calibrating the FTIR method. The software automatically calculates the number of factors to use in the regression analysis by calculating the predicted residual error sum of squares (PRESS) before calibrating the method. The number of factors suggested by the PRESS analysis was used in the PLS regression analysis. The mid-IR regions selected for analysis of the sugars and lignin were 3800 cm^{-1} to 2400 m^{-1} and 1846–644 cm^{-1}. The region between

Table 1
Correlation Coefficients (r), r^2, and SEE
for 34 Washed and Vacuum-Dried Pretreated Whole-Tree Forest Thinnings
and Southeast Alaska Softwood Residues Determined
by PLS Regression Analysis

Component	Correlation coefficient (r)	r^2	SEE (wt%)
Glucose	0.9552	0.9125	4.4
Mannose	0.9736	0.9479	[a]
Galactose	0.9110	0.8299	[a]
Xylose	0.9303	0.8655	[a]
Arabinose	0.5333	0.2844	[a]
Lignin	0.9934	0.9868	2.0

[a]SEE values less than the 1.5 wt% variability in wet chemical solids compositional analysis.

1900 and 2200 cm^{-1} was avoided because of the very strong diamond IR absorption. The regression was performed on the entire set of 35 spectra, except one sample (chosen randomly by the software) was not used in the calibration. This sample was used instead as a validation standard for the PLS method. In addition, the regression analysis was cross-validated using the leave-one-out regression analysis option in the software package. The leave-one-out cross-validation regression generally gave poorer correlation of predicted vs measured values; however, the correlation coefficients were >0.9 (e.g., the correlation coefficients in Table 1 for glucose decreased from 0.9552 to 0.9392 and lignin decreased from 0.9934 to 0.9759). The lower correlation for the leave-one-out cross-validation is the result of the limited number of samples for which wet chemical analyses are available. Results from the first PLS regression analysis (not the leave-one-out analysis) were used to plot the FTIR predicted wt% compositional values vs the measured solid compositional analyses.

Results and Discussion

Figure 1 shows the PLS regression analysis for glucose of 34 washed and 45°C vacuum-dried first- and second-stage pretreated whole-tree forest thinnings and southeast Alaska softwood residues. Figure 2 shows the PLS regression analysis for lignin of the same 34 washed and 45°C vacuum-dried samples.

Table 1 lists the correlation coefficients (r), r^2, and root mean square SEE for 34 washed and vacuum-dried pretreated whole-tree forest thinnings and southeast Alaska softwood residues determined by PLS regression analysis. The correlation coefficients for galactose, xylose, and arabinose are low (<0.9) because the compositional values that were measured for each solid residue were low and near the detection limit for our FTIR-ATR instrument and the wet chemical methods. In addition, little variance in the calibration set is expected when the results are less than the wet chemical error of ~1.5% for solids compositional analysis, resulting in
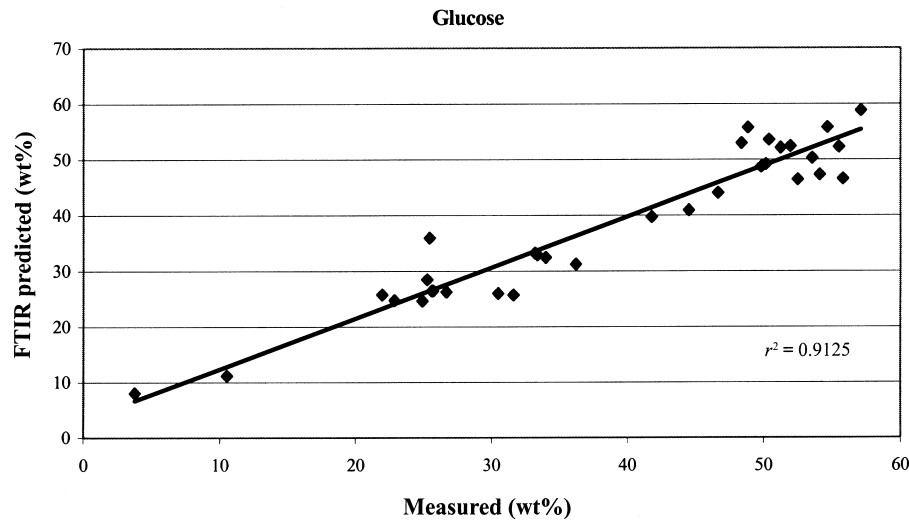
Glucose

Fig. 1. PLS regression analysis for glucose of 34 washed and 45°C vacuum-dried first- and second-stage pretreated whole-tree forest thinnings and southeast Alaska softwood samples.
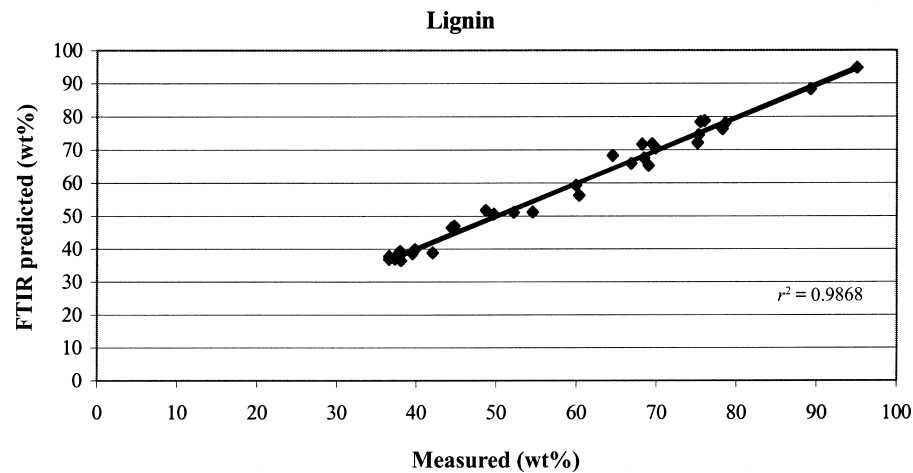
Lignin

Fig. 2. PLS regression analysis for lignin of 34 washed and 45°C vacuum-dried first- and second-stage pretreated whole-tree forest thinnings and southeast Alaska softwood samples.

little correlation of predicted values to measured values. The wet chemical solid compositional analysis on the 34 dried pretreated samples ranged from 3.77 to 57.09% for glucose, 0.06 to 2.91% for mannose, 0 to 2.87% for galactose, 0 to 7.87% for xylose, 0 to 1.94% for arabinose, and 36.58 to 95.04% for lignin. The number of factors used in the PLS regression analysis shown in Table 1 is two used for glucose, nine for mannose, nine for galactose, nine for xylose, one for arabinose, and five for lignin. The SEE for glucose
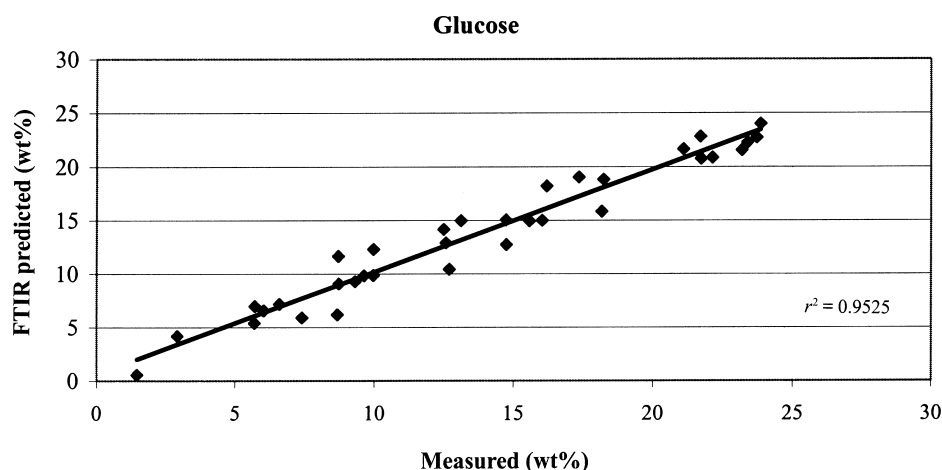
Glucose

Fig. 3. PLS regression analysis for glucose from 35 wet washed solid residues of pretreated softwood feedstocks.
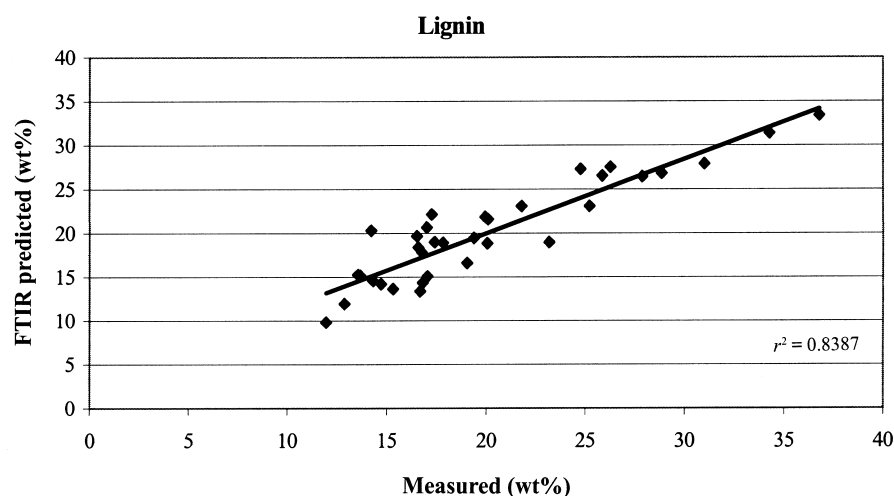
Lignin

Fig. 4. PLS regression analysis for lignin from 35 wet washed solid residues of pretreated softwood feedstocks.

reported in Table 1 is >2 wt% because the wet chemical compositional analysis for pretreated softwood residues pretreated under severe conditions is inaccurate owing to the large amount of extractives in the feedstock and degradation products produced.

Figure 3 shows the PLS regression analysis for glucose of 35 wet washed first- and second-stage pretreated softwood samples (both whole-tree forest thinnings and southeast Alaska sawdust feedstocks). Figure 4 shows the PLS regression analysis for lignin of the same 35 wet washed samples. Solids compositional data for each sample in the calibration set were adjusted for moisture before entering the data into the PLS spreadsheet.

Table 2
Correlation Coefficients (r), r^2, and SEE
for 35 Washed (Wet) Pretreated Whole-Tree Forest Thinnings
and Southeast Alaska Softwood Residues Determined
by PLS Regression Analysis of FTIR-ATR Calibration Set

Component	Correlation coefficient (r)	r^2	SEE (wt%)
Water	0.9030	0.8154	3.5
Glucose	0.9760	0.9525	1.4
Mannose	0.9706	0.9421	[a]
Galactose	0.7392	0.5464	[a]
Xylose	0.7624	0.5813	[a]
Arabinose	0.6781	0.4598	[a]
Lignin	0.9158	0.8387	2.5

[a]SEE values less than the 1.5 wt% variability in wet chemical solids compositional analysis.

The percentage of moisture was determined in triplicate on each sample by taking aliquots of the wet solids used for collecting the FTIR spectra and drying them in an oven overnight at 105°C.

Table 2 lists the correlation coefficients (r), r^2, and root mean square standard error of the estimate (SEE) for compositional analysis of 35 wet washed pretreated whole-tree forest thinnings and southeast Alaska softwood residues determined by PLS regression analysis of the FTIR-ATR calibration set. The closer the value r^2 is to 1, the higher the probability that the FTIR predicted value (y-axis) is related to the measured solids compositional value for that component (x-axis). The correlation coefficients for galactose, xylose, and arabinose reported in Table 2 are <0.9 because the solid compositional values measured for each solid residue are low and near the detection limit for this FTIR-ATR instrument and wet chemical solids compositional analysis. This is the result of hydrolysis of the hemicellulosic sugars in the feedstock resulting in low values for these three sugars in the residues. In addition, dilution by water in the wet residues further reduces the effective concentration of all components in the residues. The low correlation coefficients (<0.9) for these three sugars may be the result of little variability in the wet chemical solids compositional analysis (~1.5 wt%). The wet chemical compositional analysis on the 35 pretreated samples (corrected for moisture) in Table 2 ranged from 0.02 to 1.28% for mannose, 0 to 1.07% for galactose, 0 to 2.94% for xylose, and 0 to 0.89% for arabinose. The factors calculated from the PRESS analysis used in the PLS regression analysis for the individual components in Table 2 are as follows: one factor was used for water, five for glucose, eight for mannose, three for galactose, four for xylose, four for arabinose, and three for lignin.

Method validation requires that an analytical method have precision and accuracy. An FTIR spectrometer is expected to have a frequency error of approx 0.01 cm^{-1} *(2)*. The intensity (absorbance) error using the Nicolet Avatar 360 FTIR-ATR instrument combination was determined to have a

standard deviation of 0.002 absorbance units (au) for eight replica spectra obtained from a typical dry pretreated solid residue for a major absorbance band at 1032 cm^{-1}. These eight replica spectra were obtained following collection of 512 scans each. The sample was not removed from the cell or disturbed in order to test the spectrophotometric and software reproducibility. Each replicate spectrum was baseline corrected by drawing a baseline from 1850 to 915 cm^{-1}. The absorbance for the major absorption band at 1032 cm^{-1} was measured using the peak height tool in the Omnic® (version 5.2) software package. In addition, spectra from three individual aliquots of the same sample were obtained to test the sampling error on pretreated biomass residues. The intensity error found was 0.004 au for the major band at 1032 cm^{-1} between the three spectra. If an obvious inhomogeneity in the ground sample is chosen (e.g., a splinter that was not pretreated), an absorbance error of 0.028 au or greater is found. This demonstrates that the sample must be ground to a homogeneous mixture before placing a sample in the diamond cell. The root-mean-square of the noise of this FTIR-ATR combination was determined to be 0.00054 au in the region between 2800 and 2600 cm^{-1} using a standard software tool in the Omnic software.

The six-reflection diamond-composite cell used in this study has an apparent lower quantitation limit *(20)* of approx 2 wt% for glucan with this FTIR-ATR spectrometer combination if a 10:1 signal-to-noise ratio is used. This limit is directly related to the variance in the wet chemical method (~1.5 wt%) used for the calibration of the PLS regression method and can be improved only if a more accurate method of solid compositional analysis is developed. Models of these cells with higher number of reflection are commercially available and could extend the limits of component detection in the wet solids to lower levels, unless the background IR absorption by water in the wet washed solids increases sufficiently to interfere with the analysis.

Conclusion

In this study, we used FTIR-ATR to obtain mid-IR spectra on washed solid residues from first- and second-stage pretreated whole-tree forest thinnings and softwood sawdusts from southeastern Alaska. Spectra from 35 wet washed pretreated solid samples were subjected to PLS regression analysis, as reported in Table 2. The correlation coefficients for the regression analyses for glucose and lignin were found to be >0.9, with SEE of 1.4 and 2.5 wt%. The correlation coefficients for galactose, xylose, and arabinose were low because little variance in the calibration set is expected when composition is less than the wet chemical error of ~1.5%, resulting in little correlation of predicted values to measured values. The moisture contents of the washed solid residues in this calibration set varied between 50 and 80%. The large amount of moisture in the residues would have made obtaining mid-IR spectra very difficult because of the high background

absorbance caused by water; however, the FTIR-ATR technique was able to overcome this difficulty. The method is capable of predicting moisture content of unknown wet washed pretreated softwood residues.

The PLS regression analyses for 34 washed and 45°C vacuum-dried solid residues from first- and second-stage pretreated whole-tree forest thinnings and softwood sawdusts from southeastern Alaska are reported in Table 1. The correlation coefficients for the regression analyses for glucose, mannose, galactose, xylose, and lignin were found to be >0.9, with root-mean-square SEE varying between 2 and 4.4 wt%. The moisture contents of the 45°C vacuum-dried solid residue samples in this calibration set were measured at <1%. The wet washed samples can be generated rapidly by centrifugation, whereas the 45°C vacuum-drying step takes 3 d. Similar results are obtained if 105°C dried residues are used (data not shown). FTIR spectroscopy can be accomplished in a couple of minutes, and solids composition can be predicted rapidly once the method has been calibrated.

The FTIR-ATR technique allows mid-IR spectra to be rapidly obtained on whole slurries, washed solids, and dried washed solids from pretreated biomass. The pretreated softwood slurries used in this study were obtained from a very complex feedstock consisting of chipped whole trees, including limbs, bark, and needles. PLS methods generated using higher temperature and pressure probes in a reactor under process conditions should allow extension of this technology to rapidly monitor and control a pretreatment reactor to maximize product yield. Larger databases of FTIR spectra and solids compositional analysis will increase the accuracy of predicting the composition of pretreated solids with this method. The results presented in this study for SEE are close to the 1.5 wt% errors expected for the standard wet chemical method for solids compositional analysis. However, methods developed using PLS for one feedstock should be redeveloped when changing to other feedstocks.

Acknowledgments

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